Effect of dietary α-linolenate/linoleate balance on brain lipid compositions and learning ability of rats

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Abstract Spontaneously hypertensive rats (SHR) and normotensive control, Wistar/Kyoto (WKY) rats through two generations were fed a semipurified diet supplemented either with safflower oil (rich in linoleate) or with perilla oil (rich in α-linolenate). The cerebral lipid contents and phospholipid compositions did not differ between the two dietary groups of SHR rats. There were also no differences in the unsaturated/saturated ratios of individual phospholipids or the proportions of plasmalogens. However, the proportions of (n-3) and (n-6) fatty acids were significantly different. Decreases in the proportions of docosahexaenoate [22:6 (n-3)] in phosphatidylethanolamine and phosphatidylserine in the safflower oil group were compensated for with increases in the proportions of docosatetraenoic [22:4 (n-6)] and docosapentaenoic [22:5 (n-6)] acids as compared with the perilla oil group. These differences in phospholipid acyl chains were much smaller than the difference in the proportions of linoleate and α-linolenate of the diets. In a brightness-discrimination learning test, the total number of responses to the positive and negative stimuli were less in the groups fed perilla oil. However, the α-linolenate-deficient group took longer to decrease the frequency of R^- responses and therefore longer to learn the discrimination. Consequently, the correct response ratios were higher in the perilla oil groups than in the safflower oil groups. Thus, the dietary α-linolenate/linoleate balance influenced the (n-3)/(n-6) balance of polyenoic fatty acids differently among brain phospholipids. These changes in fatty acid composition were accompanied by changes in the brightness-discrimination learning ability in SHR and WKY rats, with rats fed a diet enriched in α-linolenate being superior in the correct response ratio. — **Yamamoto, N., M. Saitoh, A. Moriuchi, M. Nomura, and H. Okuyama.** Effect of dietary α-linolenate/linoleate balance on brain lipid compositions and learning ability of rats. *J. Lipid Res.* 1987. **28:** 144-151.

Supplementary key words safflower oil • perilla oil • brain phospholipids

Animal cells in culture generally do not require polyenoic fatty acids for growth or division. However, the deprivation of polyenoic fatty acids from the diet produces an essential fatty acid (EFA)-deficiency in animals. The major, easily recognizable symptoms of EFA-deficiency are skin lesions and growth retardation (1-3). Dietary supplementation with linoleate [18:2 (n-6)][1] prevents such symptoms completely. Furthermore, it has been reported that rats can be raised through three generations by supplementing only with linoleate without apparent deficiency symptoms (2). Recent studies with prostaglandin autacoids derived from (n-6) eicosapolyenoic acids have further served to establish the essentiality of the linoleate (n-6) series fatty acids for animal nutrition. In contrast, dietary supplementation with α-linolenate prevents only some of the symptoms of the essential fatty acid deficiency (1-3). Prostaglandin synthesis from (n-3) series fatty acids has been recognized in some tissues (4-7), but the specific physiological activities of the derivatives tended to be lower than the (n-6) derivatives. No specific functions have been assigned for eicosanoids derived from (n-3) series fatty acids. Thus, there is a question of whether α-linolenate is an essential nutrient for higher animals (8).

Docosahexaenoic acid [22:6 (n-3)] is known to be one of the major unsaturated fatty acids of phospholipids in such tissues as brain, adrenal, and muscle (9). Several research groups have reported that α-linolenate is essential for the functions of these tissues. Fiennes, Sinclair, and Crawford (10) observed skin lesions and mental disorders in monkeys, Futterman, Downer, and Hendrickson (11) and Benolken, Anderson, and Wheeler (12) have described a smaller amplitude in the electroretinogram, Connor et al. (13) have described lower visual acuity thresholds, and Lamptey and Walker (14) have reported inferior learning capacities associated with α-linolenate deficiencies. In the study of Lamptey and Walker (14), learning capacity was estimated by a simple Y maze test

Abbreviations: EFA, essential fatty acids; SHR, spontaneously hypertensive rats; WKY, Wistar/Kyoto rats; CRF, continuous reinforcement.

[1]Fatty acids are abbreviated by the carbon chain length:the number of double bonds. The position of the first double bond numbered from the methyl terminus is designated as (n-9), (n-6), or (n-3).

in rats fed either safflower oil or soy bean oil as the source of essential fatty acids. In repeating and extending their work, Bowman and Davenport (15) found no difference in the learning capacity in X maze discrimination between soybean oil- and safflower oil-fed rats. In this report, we have attempted to determine whether there is a correlation in learning ability of rats with the relative dietary intake of α-linolenate or linoleate. Learning ability was analyzed using an apparatus which appears to allow a more objective estimate (16, 17). Rats were fed two different vegetable oils that vary markedly in α-linolenate/linoleate ratios, but contain similar proportions of saturated and monoenoic fatty acids.

MATERIALS AND METHODS

Animals

Spontaneously hypertensive rats (SHR) and normotensive Wistar/Kyoto rats (WKY) (Charles River of Japan Co.) were used. Female and male rats at 4 weeks of age were fed a semi-purified diet for 7 weeks, prior to mating and then throughout pregnancy and lactation. The basal diet (Oriental Yeast Co., Ltd., Tokyo) contained by weight, 39% corn starch, 25% milk casein, 10% alpha-starch, 8% cellulose powder, 6% minerals,[2] 5% sucrose, 2% vitamin mixture,[3] and 5% oil. Safflower oil and perilla oil were used. Perilla oil extracted from perilla seeds (*Perilla frutescens* var. *crispa*) was assessed for edibility by measuring peroxide value, polychlorinated benzene content, cyanide content, and heavy metals content. For the analysis of brain lipids, rats fed a conventional diet (Oriental Yeast Co., MF)[4] were also examined. The fatty acid compositions of the diets are shown in **Table 1.** The male pups were weaned to the diets consumed by the dams and were fed those diets. These rats (F_1) at 11 weeks of age, when their daily food intake became roughly constant, were used for the learning test.

Lipid analysis

After the learning test, rats were killed by decapitation and the cerebral right hemispheres were removed, frozen quickly with dry ice, and stored in liquid nitrogen. Samples were thawed and lipids were extracted twice with chloroform–methanol according to the method of Bligh and Dyer (18). Phospholipids were separated by two-dimensional thin-layer chromatography on prewashed and activated silica gel plates (Merck 60). The developing solvents were chloroform–methanol–28% NH_4OH 60:40:6 for the first dimension and chloroform–acetone–methanol–acetic acid–water 50:20:10:15:5 for the second dimension. Spots were located by spraying first with Rhodamin 6G solution and then with 28% NH_4OH. Lipids were extracted from the corresponding spots twice with chloroform–methanol–3% NH_4OH 6:5:1 and then once with chloroform–methanol 2:1. Heptadecanoic acid was added as an internal standard. The combined extracts were evaporated to dryness with nitrogen gas. Fatty acids were converted to methyl esters by treating with 5% HCl in methanol and were analyzed by gas-liquid chromatography.

TABLE 1. Fatty acid composition of vegetable oil-supplemented diets

Fatty Acid	Safflower Oil Diet	Normal Diet	Perilla Oil Diet
	% of total fatty acids		
14:0	0.5	0.5	0.6
16:0	8.6	15.5	8.1
16:1	ND	1.5	ND
18:0	2.2	2.5	1.9
18:1 (n-9)	10.4	22.8	12.3
18:2 (n-6)	78.0	48.8	12.8
18:3 (n-3)	0.05	4.1	64.0
20:1 (n-9)	ND	0.9	ND
20:3 (n-9)	ND	ND	ND
20:4 (n-6)	0.2	0.4	0.2
20:5 (n-3)	ND	1.5	ND
22:6 (n-3)	ND	1.1	ND

Lipids were extracted from the diets with chloroform–methanol. Fatty acids were converted to methyl esters and analyzed by gas-liquid chromatography. Fatty acids were designated by the carbon chain: the number of double bonds. The position of the first double bond numbered from the methyl terminus is designated as (n-9), (n-6), or (n-3). ND, not detected.

Brightness-discrimination learning test

The conditioning chamber (Skinner box, Ralph Gerbrands Co., G7010) had a recessed food magazine on its front wall and a lever near the lower right-hand corner of the same wall (19). A light screen of 4 cm diameter for presenting a positive or a negative stimulus (S^+ or S^-) was located on the front wall. The brightness of this screen was 5×10^4 foot Lambert (fL) as an S^+ stimulus and 1/1,000 brightness of S^+ as an S^- stimulus.[5] Experimental events were controlled and recorded automatically by a microcomputer.

[2]The semi-purified diet contained 420 mg of K, 990 mg of P, 560 mg of Ca, 250 mg of Na, 74.9 mg of Mg, 27.0 mg of Fe, 5.1 mg of Zn, 2.2 mg of Mn, 0.57 mg of Cu, and 0.46 mg of I.

[3]Vitamins A, D_3, B_1, B_2, B_6, B_{12}, C, E, K_3, biotin, folic acid, Ca-pantothenate, *p*-aminobenzoic acid, niacin, inositol, and choline chloride were included.

[4]The conventional diet (Oriental Yeast Co.) is made of wheat, corn, barley, fish meal, defatted milk, soy meal, yeast, vitamin and mineral mixtures, meat powder, and wheat germ.

[5]This dark light is more than 2000 times the threshold for inducing electric impulses in retina, as determined in the electroretinogram (Watanabe, Kato, Aonuma, Hashimoto, Naito, Moriuchi and Okuyama, unpublished results).

The outline of the experimental schedule is illustrated in **Scheme 1.** Food consumption of rats was controlled so as to decrease their body weight to 85% of the normal in a week. This level was maintained throughout the experiments.

Rats were trained in Skinner boxes according to a schedule of shaping on continuous reinforcement (CRF) for approximately 20 to 40 min a day, during which a bright light was shown on the front screen of the boxes. On day 1, the rat was given one pellet whenever it thrust its nose into the magazine. At the same time, when the rat touched or pressed a lever, one pellet was delivered, which was continued over a period of 40 min. On days 2 and 3, a CRF acquisition schedule was carried out, with one pellet delivered for every lever press, either for up to 20 min or until the number of reinforcements reached 40 CRF. When the total time required for 40 CRF became less than 6 min, this CRF schedule was discontinued.

After the completion of the CRF schedule, the rats were trained for variable intervals of 5 sec, on average, of the pellet reinforcement until the number reached 40 CRF. Then the brightness-discrimination learning test was started.

When the brighter light of 5×10^4 fL was given on the screen as a positive stimulus (S^+) for 20 sec, the lever-pressing responses were reinforced. When the darker light (S^-), i.e., 1/1,000 of S^+, was present, no pellet was given in response to the rat's lever pressing. Variable interval was 15 sec. Stimulus was given for 20 sec with an interval of a 5-sec blackout, and randomized according to a Gellerman (1933) sequence (20). One session consisted of 20 times each of S^+ and S^- (20 S^+ and 20 S^-), and the session was run every day. Correct response ratio, $R^+/(R^+ + R^-) \times 100$, was calculated from the number of correct responses (R^+) during S^+ presentation and that of incorrect responses (R^-) during S^- presentation in a session (21). It should be noted that a false negative response corresponding to the failure of rats to depress the lever in response to S^+ stimulus was not included in these calculations.

RESULTS

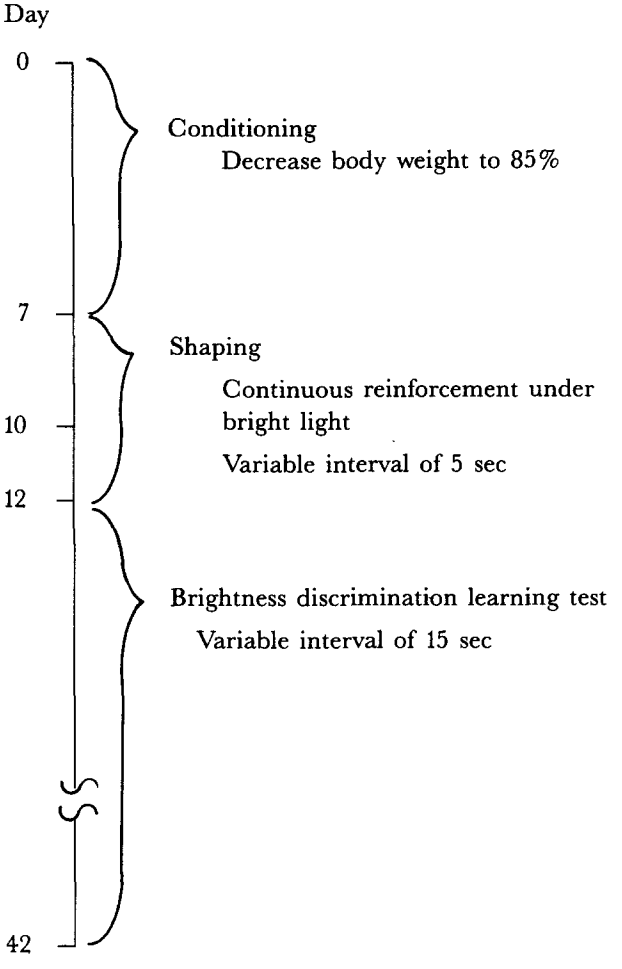
In the two diet groups, the weight gain and the litter size were not significantly different.

Lipids extracted from cerebral right hemispheres of SHR rats fed either the safflower oil diet or perilla oil diet through two generations were analyzed. The lipid contents and phospholipid compositions were not significantly different between the two dietary groups (**Table 2**). The plasmalogen content (as determined by dimethylacetal content), the proportions of saturated fatty acids, and the unsaturated/saturated ratios of the individual lipid classes were changed relatively little. However, the proportions of (n-3) and (n-6) polyenoic fatty acids varied significantly between the two dietary groups. The major differences in the acyl chains were seen in the phospholipid fractions. Data for Wistar rats fed a normal diet are also included as a control, but the basal diet was a commercial diet that differed from the semi-purified diet used for the supplementations with safflower oil or perilla oil.

The relative lack of α-linolenate [18:3 (n-3)] in the safflower oil diet led to a decrease of 22:6 (n-3) in phosphatidylethanolamine and phosphatidylserine; however, there was a compensatory increase in 22:4 (n-6) and 22:5 (n-6), two fatty acids that can be produced from linoleate. The proportions of polyenoic fatty acids in phosphatidylcholine were relatively small, but dietary effects similar to those seen in phosphatidylethanolamine and phosphatidylserine were observed. It was surprising that the proportions of 22:6 (n-3) in these phospholipids were very similar in the normal diet group and perilla oil diet group, despite a tremendous difference in the proportions of α-linolenate in the two diets (4% vs. 64% of the total). In contrast, only a small amount of (n-3) fatty acid was found and arachidonate [20:4 (n-6)] was conserved highly selectively in phosphatidylinositol; the difference in the proportions of 18:2 (n-6) in the safflower oil diet and perilla oil diet (78% vs. 13% of the total) did not affect the proportion of 20:4 (n-6) in the phosphatidylinositol fraction significantly. The proportion of octadecenoate (18:1) in phospholipids was higher in the perilla oil diet group than in the safflower oil diet group.

In the shaping process of the learning test, all trained rats could obtain 40 reinforcement pellets within 6 min after 5 days. After this process, rats were subjected to a brightness-discrimination learning test. As shown in **Fig. 1,** the total response ($R^+ + R^-$) increased along with the progress of the session similarly in the SHR rats fed either safflower oil diet or perilla oil diet for the initial 15 sessions. Thereafter, the total response in the safflower oil group continued to increase, but that in the perilla oil group tended to decrease. This difference in the total responses is due to the differences in both the correct response (R^+) and the incorrect response (R^-). The R^+ response continued to increase throughout the session in the safflower oil group whereas the rate of increase in R^+ was smaller after the 12th session in the perilla oil group.

On the other hand, the R^- response increased similarly to R^+ response in the two groups during the first several sessions. Thereafter, the α-linolenate-deficient group took longer to decrease the frequency of R^- responses and, therefore, longer to learn the discrimination. In the safflower oil group, the decrease began at the 16th session, while in the perilla oil group it began at the 9th session. This difference in R^- response was more distinct than that in R^+ response. As a consequence of these differences, the correct response ratio was higher in the perilla oil group than in the safflower oil group in SHR rats (**Fig. 2**).



Scheme 1. Schedule for brightness-discrimination learning test.

Qualitatively similar but more pronounced differences were observed in the WKY rats (the normotensive control) (**Fig. 3**).

The total response was higher in the safflower oil group than in the perilla oil group. The R^+ response was similar in both groups for up to the 20th session, but thereafter it was slightly lower in the perilla oil group. The major difference was observed in the R^- response; it tended to decrease clearly after the 20th session in the safflower oil group as compared to the 9th session in the perilla oil group. This difference in the R^- response was mainly reflected in the difference in the total responses described above. Again, the correct response ratio was higher in the perilla oil group than in the safflower oil group after the 13th session (Fig. 2b). The difference in the correct response ratios of the two groups was statistically significant ($P < 0.01$) as examined by two-way analysis of variance.

DISCUSSION

Since the extensive studies on the effects of dietary α-linolenate/linoleate balance on brain lipids by Mohrhauer and Holman (22), many workers have dealt with related problems. Brown, Marshall, and Johnston (23) have described the fatty acids of the individual phospholipids in the most detail. The combination of safflower oil and perilla oil used in the present experiments has not been tested previously in a nutritional study. Perilla oil has the highest α-linolenate/linoleate ratio and safflower oil has the lowest ratio among the available vegetable oils. Although only 13% of the total fatty acid in the perilla oil diet was linoleate, no symptoms of essential fatty acid deficiency were apparent in rats maintained by feeding the perilla oil diet through three generations. The differences in the fatty acid compositions of major phospholipids, phosphatidylcholine and phosphatidylethanolamine, were qualitatively similar to those described earlier in studies using different combinations of oils (22, 23). However, two interesting features were found in the analyses of phosphatidylinositol and phosphatidylserine. First, the dietary-induced changes in the fatty acid composition of phosphatidylserine were similar to those of phosphatidylethanolamine, and second, there was a highly selective conservation of 20:4 (n-6) in phosphatidylinositol. Enzymes involved in the metabolism of these phospholipids in brain must be selective enough to retain 22:6 (n-3) in phosphatidylethanolamine and phosphatidylserine and 20:4 (n-6) in phosphatidylinositol. The elongation and desaturation systems must be involved in these changes in the fatty acid patterns. It is generally accepted that the preference of these systems for these fatty acids is in the order of (n-3), (n-6), and (n-9) series (8, 22). However, the relative contributions of these systems in different organs, e.g., brain and liver, remain to be elucidated.

Lamptey and Walker (14) first reported the inferior learning ability of rats fed a safflower oil diet as compared to rats fed a soybean oil diet. In that study learning ability was determined by a simple Y maze test. However, Bowman and Davenport (15) found no difference in X maze discrimination, as critically reviewed by Bivins et al. (8). In the brightness-discrimination learning test which, we believe, allows more objective estimates of the learning ability, we found that the correct response ratios were inferior in rats fed diets deficient in α-linolenate (Fig. 2). Furthermore, we found that the major effect of α-linolenate deficiency is in the response to new conditions; the α-linolenate-deficient group took much longer to decrease the frequency of R^- response and therefore longer to learn the discrimination (Figs. 1 and 3). These observations, together with the effects of α-linolenate deficiency on the functions of retinal function (11-13)[5] and

TABLE 2. Effect of the dietary α-linolenate/linoleate balance on the profile of polyenoic fatty acids in cerebral phospholipids from spontaneously hypertensive rats

	Phosphatidylethanolamine			Phosphatidylcholine			Phosphatidylinositol		Phosphatidylserine		
Fatty Acids	S	N	P	S	N	P	S	P	S	N	P
14:0	tr.	tr.	tr.	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.4 ± 0.2	tr.	0.1 ± 0.0	tr.
14:1	tr.	tr.	tr.	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	0.3 ± 0.1	tr.	0.2 ± 0.0	tr.
16:0 DMA[a]	7.8 ± 0.3	5.4 ± 0.7	8.0 ± 0.2	0.3 ± 0.0	0.1 ± 0.1	0.4 ± 0.0	tr.	tr.	0.1 ± 0.0	tr.	tr.
16:0	5.0 ± 0.2	6.0 ± 0.3	4.7 ± 0.2	43.6 ± 1.5	44.5 ± 1.5	42.0 ± 0.7	9.6 ± 0.3	9.5 ± 0.8	1.1 ± 0.1	1.4 ± 1.2	0.9 ± 0.0
16:1	0.6 ± 0.0	0.7 ± 0.1	0.7 ± 0.1	1.1 ± 0.1	0.3 ± 0.2	1.1 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
18:0 DMA	10.4 ± 0.1	6.7 ± 2.1	10.5 ± 0.4	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	tr.	tr.	tr.	tr.	0.1 ± 0.0
18:0	22.6 ± 0.2	27.0 ± 0.7	22.3 ± 0.3	12.5 ± 0.3	12.8 ± 0.1	12.8 ± 0.1	46.4 ± 1.4	42.9 ± 1.6	44.7 ± 0.6	49.7 ± 2.7	42.3 ± 0.9
18:1	15.3 ± 0.3	14.7 ± 0.2	18.0 ± 0.2**	27.2 ± 0.4	26.2 ± 0.4	28.7 ± 0.3*	4.1 ± 0.1	4.9 ± 0.5	19.1 ± 0.6	17.8 ± 0.3	20.9 ± 0.2*
18:2 (n-6)	0.3 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	0.9 ± 0.0	1.1 ± 0.1	0.9 ± 0.1	0.2 ± 0.1	1.5 ± 1.3	0.1 ± 0.1	0.2 ± 0.0	0.1 ± 0.0
18:3 (n-6)	0.1 ± 0.0	tr.	0.1 ± 0.0	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
18:3 (n-3)	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.0	0.4 ± 0.2	0.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.3 ± 0.2	0.4 ± 0.0	0.4 ± 0.1	0.4 ± 0.0
20:1	2.6 ± 0.0	2.6 ± 0.4	2.7 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	1.4 ± 0.0	1.4 ± 0.1	1.3 ± 0.0*
20:3 (n-9)	0.2 ± 0.1	0.6 ± 0.5	0.1 ± 0.0	0.4 ± 0.2	0.3 ± 0.1	0.2 ± 0.0	tr.	tr.	0.4 ± 0.1	0.2 ± 0.1	0.2 ± 0.0
20:3 (n-6)	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	tr.	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.0
20:4 (n-6)	12.3 ± 0.3	11.7 ± 0.4	9.3 ± 0.1***	6.0 ± 0.2	5.6 ± 0.4	5.0 ± 0.1*	37.4 ± 0.7	35.8 ± 2.3	4.2 ± 0.1	3.7 ± 0.2	3.7 ± 0.1*
20:4 (n-3)	tr.	0.1 ± 0.0	0.1 ±	0.4 ± 0.3	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.9 ± 0.2	0.6 ± 0.1	0.3 ± 0.3
20:5 (n-3)	tr.	0.2 ± 0.2	0.2 ± 0.0*	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	tr.	0.2 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.2 ± 0.1
22:4 (n-6)	6.5 ± 0.0	4.4 ± 0.3	3.8 ± 0.1***	1.2 ± 0.1	0.6 ± 0.1	0.5 ± 0.1*	0.3 ± 0.2	0.3 ± 0.1	4.1 ± 0.2	2.3 ± 0.3	2.6 ± 0.3**
22.5 (n-6)	7.8 ± 0.2	0.2 ± 0.1	0.1 ± 0.1***	2.1 ± 0.1	0.8 ± 0.3	0.1 ± 0.1***	0.2 ± 0.1	tr.	12.7 ± 0.5	0.6 ± 0.3	1.4 ± 0.2***
22:5 (n-3)	tr.	tr.	0.9 ± 0.1***	tr.	0.1 ± 0.1	0.5 ± 0.3	tr.	0.1 ± 0.1	tr.	tr.	0.9 ± 0.3*
22:6 (n-3)	7.4 ± 0.7	16.4 ± 1.3	17.3 ± 0.2***	1.4 ± 0.2	3.6 ± 0.5	4.5 ± 0.7*	0.3 ± 0.2	2.1 ± 0.2**	8.1 ± 0.4	20.4 ± 2.4	22.4 ± 0.5***
Total (n-6)	27.3	16.9	13.7	10.3	8.3	6.7	38.1	37.7	21.3	7.0	8.1
Total (n-3)	7.5	16.8	18.7	2.3	4.2	5.6	0.5	2.8	9.8	21.6	24.3
Total fatty acids (μg/100 mg)	780.6 ± 65.5	ND	785.2 ± 11.2	710.8 ± 9.8	ND	664.4 ± 17.1	50.5 ± 5.1	51.7 ± 4.9	259.5 ± 33.0	ND	256.2 ± 17.5

Spontaneously hypertensive rats were fed a semi-purified diet supplemented with either safflower oil (S) or perilla oil (P) through two generations. Wistar rats fed a conventional diet (N) were used as controls. Fatty acids were analyzed by gas–liquid chromatography. For the S and P diets, averages of three determinations (three rats) are presented, while averages of two determinations each assayed in duplicate are presented for diet N. The maximal deviation from the mean was 5% of the values given for the major fatty acids. Statistical significance between the perilla oil group and the safflower oil group is shown: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. ND, not determined; tr., trace.

[a]DMA, dimethylacetal.

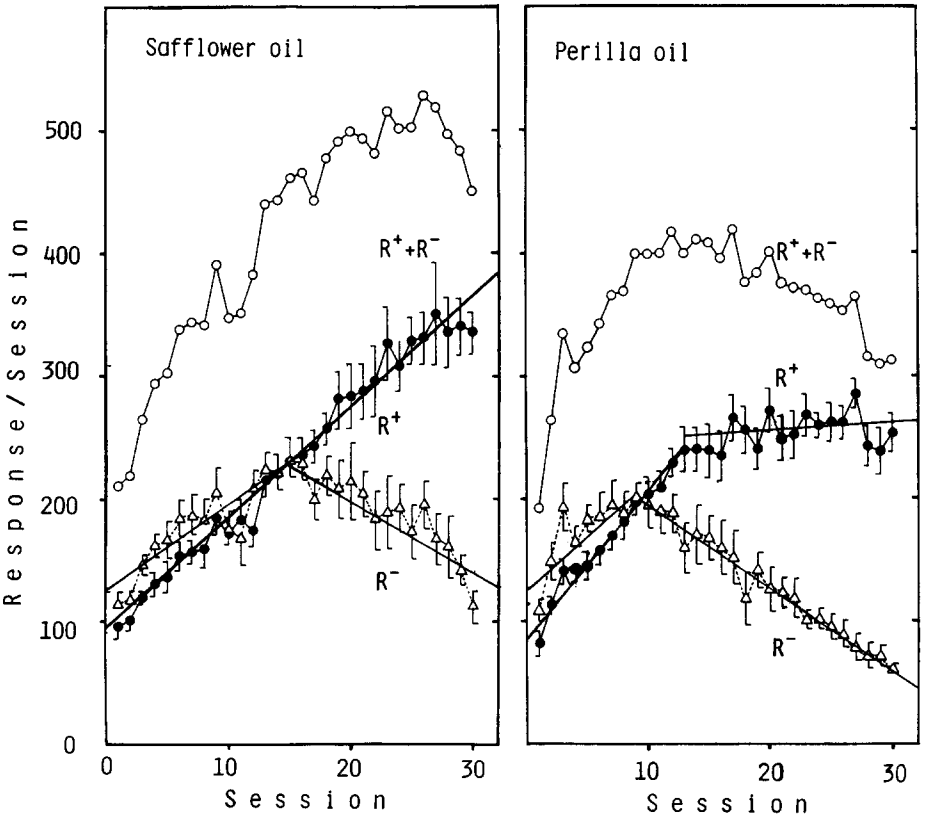


Fig. 1. Brightness-discrimination learning test in spontaneously hypertensive rats (SHR) fed either a safflower oil diet or a perilla oil diet. SHR rats fed the safflower oil diet or the perilla oil diet for two generations were first trained at 11 weeks of age in a Skinner box to be reinforced to receive pellets upon pressing a lever. Then the brightness-discrimination learning test was begun. When the brighter light (5×10^4 fl) was shown on the screen as a positive stimulus (S^+) for 20 sec, the lever-pressing responses (R^+) were reinforced. When the darker light (S^-) was present, no pellet was given in response to level pressing (R^-). This dark light is more than 2000 times the threshold for inducing electric impulses in retina, as determined in the electroretinogram (unpublished results). Stimulus was given for 20 sec with an interval of a 5-sec blackout, and randomized. One session consisted of 20 times each of S^+ and S^-, and the session was run every day. Each group consisted of 10 rats and averages of 10 determinations (± SEM) are presented. (○), Total response ($R^+ + R^-$); (●), correct response (R^+); (△), incorrect response (R^-).

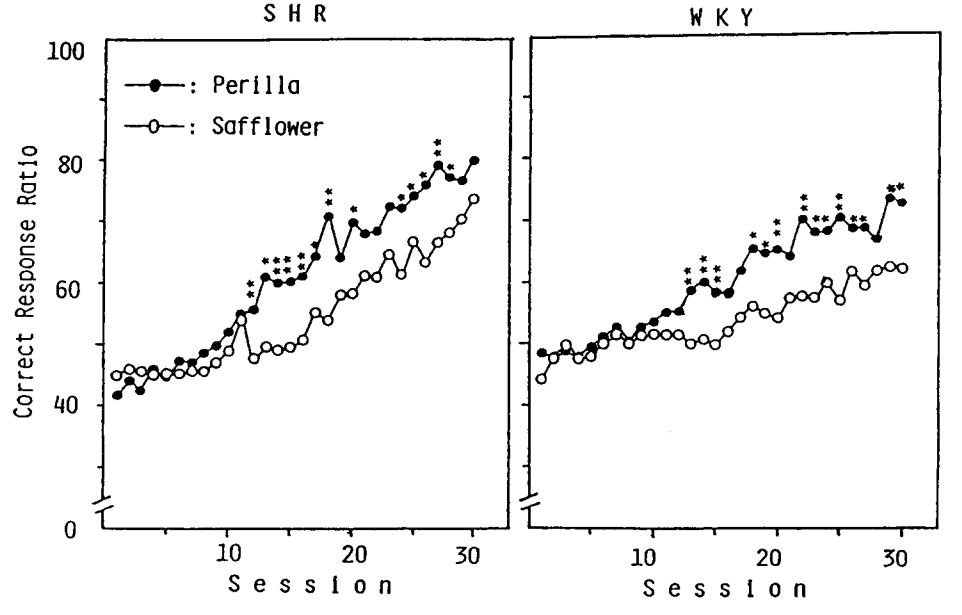


Fig. 2. Correct response ratio in the brightness-discrimination learning test. The correct response ratio, $R^+/(R^- + R^-) \times 100$, was calculated from the data presented in Figs. 1 and 3; *, $P < 0.05$; **, $P < 0.01$.

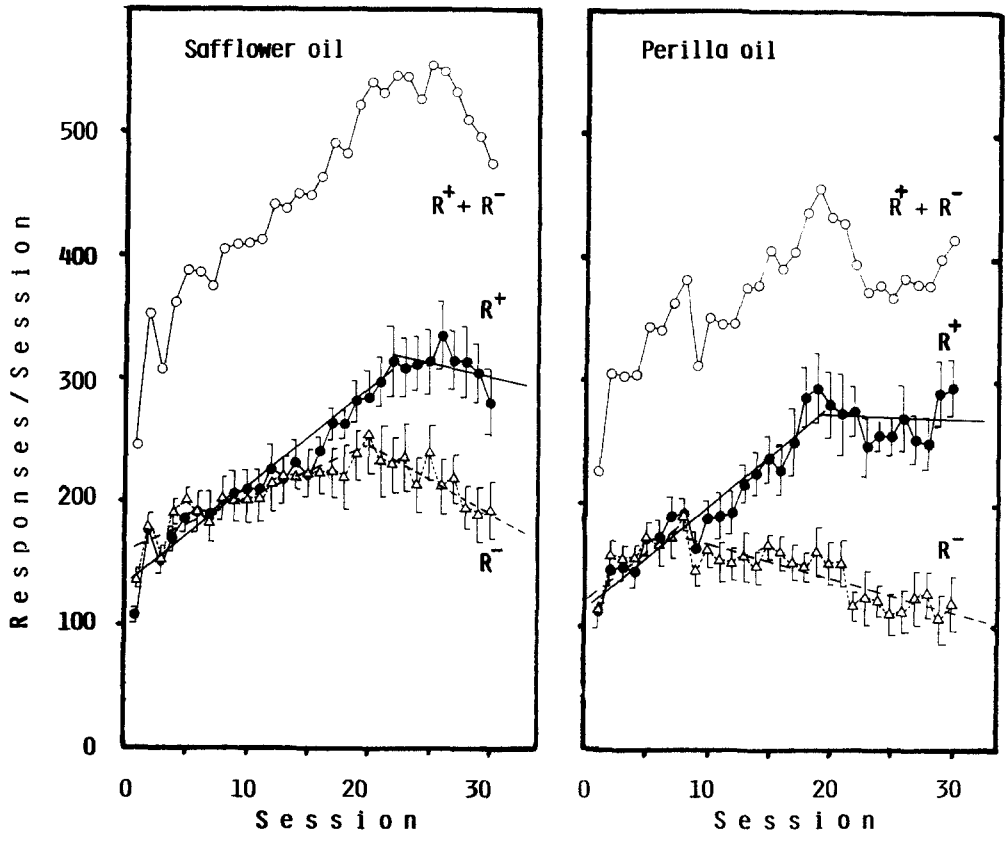


Fig. 3. Brightness-discrimination learning test in Wistar/Kyoto rats. The experimental conditions were the same as in Fig. 1, except that normotensive control Wistar/Kyoto (WKY) rats were used. Averages of 10 determinations (10 rats) ± SEM are presented.

skin properties (10), support the conclusion that α-linolenate is essential for higher animals; this has been critically reviewed (8).

Recently, case reports were published which suggested that α-linolenate is essential for nerve functions in humans (24, 25). Either autocoids may be formed from (n-3) fatty acids, or (n-3) fatty acids may regulate the metabolism of (n-6) fatty acids (23, 26). In fact, Brown et al. (23) have shown that prostaglandin $F_{2\alpha}$ synthesis in brain slices was less in a linseed oil-fed group than in a corn oil-fed group. In this context, the sleep-inducing activity of prostaglandin D_2 and the presence of its receptor in brain (27) seem to be quite interesting, since neurotic disorders usually accompany insomnia. The molecular mechanisms of the essentiality of α-linolenate and the roles of eicosanoids in the functions of nervous tissues remain to be elucidated.

Very recently, the possible existence of a food-mood link has been discussed (28). Although this latter article did not discuss the problems of essential fatty acids, higher animals depend entirely on their diets for linoleate and α-linolenate. Various diets as well as different vegetable oils contain quite different proportions of linoleate and α-linolenate. Accordingly, the α-linolenate/linoleate balance in diets must be evaluated for its importance in human nutrition.

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